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Bioleaching of heavy metals from contaminated alkaline sediment by auto- and heterotrophic bacteria in stirred tank reactor

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Abstract: Bioleaching Xiangjiang River alkaline sediment contaminated by multiple heavy metals was investigated. Multiple metals in alkaline sediment possess significant toxicity to aquatic organisms or humans and will greatly inhibit bioleaching. The bioleaching method using autotrophic bacteria mixed with heterotrophic bacteria can solve this problem successfully. The experiment results showed that bioleaching efficiencies of Zn, Mn, Cu, and Cd were 95.2 %, 94.2 %, 90.1 %, and 84.4 %, respectively. Moreover, the changes of heavy metal concentrations in different fractions in contaminated sediment before and after bioleaching were analyzed by selective sequential extraction, and it was discovered that the main fractions of Zn, Mn, Cu and Cd after bioleaching are Fe−Mn oxide, organic associated form and a residual form. Its biotoxicity decreased greatly. The bioleaching heavy metals from sediment using autotrophic bacteria combined with heterotrophic bacteria can effectively improve the bioleaching efficiency and reduce toxicity.

Key words: bioleaching; alkaline sediment; heavy metals; sequential extraction; auto- and heterotrophic bacteria

1 Introduction

With the development of metal processing, tanneries and electroplating industries [1], heavy metal contamination becomes a worldwide environmental problem. It is persistent when releasing into environment and can pose significant risks to public health and ecosystems through the contamination of food chains, breaking the normal metabolism activity of organisms [2,3]. Therefore, it is a global environmental concern to clean up the metal contaminated sites. The toxicity and mobility of metals depend not only on total concentration, but also on their specific chemical forms, so the knowledge on metal speciation may be as important as the total metal concentrations for hazard assessment studies [4]. Metals deposited in the sediment can be present in a number of chemical forms, such as exchangeable, carbonate associated, Fe−Mn oxide-associated, organic associated, and residual forms [4,5].

Bioremediation has been developed as an environmentally friendly and cost-effective technology for the removal of metals from sediments. Among the biological technologies, microbiological leaching of heavy metals from sediment is far more popular. Bioleaching is defined as "the solubilization of metals from solid substrates either directly by the metabolism of leaching bacteria or indirectly by the products of metabolism" [6]. Bioleaching process using acidophilic microorganisms (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) and neutrophilic microorganisms (*Aspergillus niger*) have been applied successfully to metal removal from sediment, municipal solid, and sludge [7−11]. Whereas these materials are commonly complex mixtures consisting mainly of organic compounds, such as organic acids, simple sugars and amino acids, which can intensively inhibit chemolithoautotrophic microbes, thus decreasing the efficiency of metal dissolution from solid substrates [9,12,13]. Heterotrophic microbe has gained

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an increasing attention since it can use the organic matter as energy to eliminate these toxic organic compounds to chemolithoautotrophic bacteria.

Xiangjiang River is a major tributary of the Yangtze River in Hunan province. A large number of non-ferrous metal and chemical enterprises, whose pollutants entering this river located in this valley, have contaminated the Xiangjiang River [14], and emissions of arsenic, chromium, cadmium, lead, and mercury in Hunan have ranked the first in China [15,16]. The basin suffered severely heavy metal pollution over a period of around 50 years. Simultaneity, the river organic pollution is an increasingly serious problem in recent years [17,18]. But little has been done about the removal of heavy metals from the highly contaminated alkaline sediments.

This work used an artificial consortium constructed by auto- and heterotrophic bacteria to improve the efficiency of removal of heavy metals from highly contaminated dredged alkaline sediments of Xiangjiang River in a stirred tank reactor, and the process was based on: firstly, inocula of heterotrophic bacteria mixture (HBM) able to oxidize organic compounds such as organic acids, simple sugars and amino acids, secondly inocula of *Pseudomonas aeruginosa* ZH able to assist the growth of sulfur-oxidizing microorganism by consuming microbial metabolites and oxidizing S^0 produced acid which is benefit for the sulfur-oxidizing microorganism growth and *Acidithiobacillus thiooxidans* DMC able to oxidize sulphur and sulfide.

2 Experimental

2.1 Contaminated sediment

The sediment for the present study was collected from the Xiawan port of Xiangjiang River located at Zhuzhou city, in September, 2010, which is an industrial town in Hunan Province. Sediment (0−10 cm in depth) was sampled and mixed completely and temporarily stored at 4 °C. Some sample was air-dried and divided into two parts, one was for bioleaching, and the other was processed with 2 mm sieve pore size for physiochemical analysis.

2.2 Sediment analysis

The selected physicochemical properties of sediment were analyzed according to the routine methods: pH of soil was measured on 1:2.5 (soil to distilled water) soil slurry. Total organic carbon (TOC) content was determined by loss-on-ignition, combusting at 450 °C for 4 h in a muffle furnace and at 105 °C for 7 h. The total concentrations of metals in sediment were analyzed by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500 series, USA). Fractionation of heavy metals present in sediment before and after bioleaching was carried out by selective sequential extraction following TESSIER et al [5] and then determined by ICP-MS.

2.3 Microorganism

This study used a procedure mixture of microorganisms, including autotrophic *A. thiooxidans* DMC, heterotrophic *P. aeruginosa* ZH, and heterotrophic bacteria mixture (HBM), which were isolated and preserved by the Key Laboratory of Biometallurgy of Ministry of Education. We used fresh sediment as the seed sediment to enrich and culture the indigenous heterotrophic bacteria added to LB medium. Until mixture resistant abilities to Zn^{2+} and Cd^{2+} were up to 1.5 g/L respectively, the inocula for the bioleaching experiments were obtained. The *P. aeruginosa* ZH and *A. thiooxidans* DMC were routinely cultivated in 9K medium with S^0 (1%, w/v), and S^0 and glucose as the energy source, respectively [19].

2.4 Bioleaching experiment

Bioleaching tests were performed in a 3 L glass cylindrical with 15 cm in inner diameter and 17 cm in height equipped with a pH controller, a temperature controller, and a stirrer. As shown in Fig. 1, the reactor was placed in a thermostatic bath to keep the temperature constant at 30 °C for 48 d. Working volume of the reactor was 1 L containing 90% (v/v) of distilled water, 5% (w/v) of contaminated sediment, and 1% (w/v) of S^0, 10% (v/v) of (100 mL) mixed proportional culture of HBM, *P. aeruginosa* ZH and *A. thiooxidans* DMC as inoculum. At first, the bacteria mud of HBM was inoculated into the tank reactor, in which the cell was approximately 1×10^6 mL^{-1}. Two days later the 100 mL equal proportional culture mixture (2×10^6 mL^{-1}) of *P. aeruginosa* ZH and *A. thiooxidans* DMC was

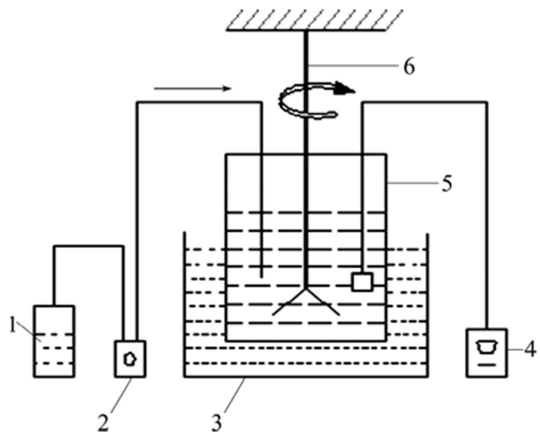

Fig. 1 Layout of bioleaching experimental apparatus (1—Distilled water; 2—Peristaltic pump; 3—Thermostat water bath; 4—pH meter; 5—Glass cylindrical reactor; 6—Stirrer)